OCT 11 1950

VOL. XI, No. 6 SEPTEMBER, 1950

10.5
873

BORDEN'S REVIEW *of* NUTRITION RESEARCH

THE PHOSPHATASE ENZYMES:
Versatile Catalysts in Nutrition

UNIV. OF MICH.

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published monthly, except July and August, by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; T. D. Waibel, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

THE PHOSPHATASE ENZYMES:
Versatile Catalysts in Nutrition

By **HALDANE GEE, Ph.D.,** ***Guest Editor****

Enzymes able to add or subtract phosphate in biochemical processes, a subject of research for fifty years, are today receiving more attention from investigators than ever before. Of the new work in the field of enzymology, the current high volume of publication on phosphatases and related biocatalysts is indicative of a trend of first importance in nutrition.

In recent years, the literature on the supply, structure and significance of the vitamins has drawn some attention away from the spectrum of the enzymes that function in the metabolism of protein, carbohydrate and fat, or that play a part in the formation of the skeleton. But *Chemical Abstracts* now carries about one-seventh as many titles on enzyme topics as it carries in the vitamin classification. Half of these enzyme titles deal with the phosphatases. They range from the complex enzyme system of carbohydrate metabolism, still being intensively investigated, to the current chemical and biophysical problem of the materials and mechanics of muscular energy, which is triggered by the enzyme adenosinetriphosphatase, probably the most important enzyme, biologically, of all.

Enzymes Differentiated from Vitamins and Hormones

There has been some understandable confusion over the respective functions of vitamins and enzymes. Less confusing, but related to a degree, is the role of the hormones. These three groups of physiologically active factors may be differentiated as accessory (the vitamins), catalytic (the enzymes), and regulatory (the hormones). They have some characteristics in common. They are essential for growth or for maintenance of health or normal bodily function. Generally they are potent, with active amounts for man measured in gammas or milligrams. They are recognized at first by their physiological effects, and are usually assumed to be protein in nature until otherwise identified, as many of them have been.

In the case of the vitamins, as the horizon was more clearly seen, objects on it were identified one after another. Those fully known have been assigned appropriate chemical names and are being thought of increasingly in terms of their specific chemical effects, rather than in terms of the over-all consequences of their omission or absence from the diet. The "vita" part of the name now connotes the living organism's need of the material, but no longer

---

*The author, formerly managing editor of *Food Industries,* is now a chemical and editorial consultant.

implies that vitamins are necessarily produced by living objects, since some of them, of course, are commercially synthesized. Significance of the "amine" appellation is now limited also since the identified accessory factors are non-protein and in many cases non-amine.

As in the case of the vitamins, the hormones have been thought of as highly organized nitrogenous substances until proven otherwise. When thyroxin was chemically identified, the presence of iodine in the molecule was the distinguishing feature, and the compound is, of course, non-protein. In the case of insulin, there seems to be no question as to the protein nature, and considerable work has been done on the way the protein molecule is put together.

Following the established pattern of these other physiological adjuncts, the enzymes, none completely identified as yet, are still regarded as proteins. Some of them have been crystallized. It may, however, later be shown that the protein is associated rather than essential.

Enzymes, and especially the phosphatase enzymes, are found in many body fluids, tissues and organs, but none has been shown of endocrine origin. Regardless of their origin, however, both synergistic and antagonistic effects between hormones and enzymes have been described. Likewise, a number of instances of inter-dependence have been well established between enzymes and vitamins. Thiamine is biologically inactive until converted into the pyrophosphate (1), when it carries the pseudo-enzyme name of cocarboxylase.

Biocatalytic Function: The distinguishing feature of the enzymes is undoubtedly their biocatalytic function, which is exercised without proportionate consumption or destruction of the accelerating agent. The catalytic effect may be obtained *in vitro*. One of the first breaks with the ancient brewing and baking arts was the production of the extra-cellular extract "zymase" from disrupted yeast cells. The resulting unorganized ferment became the first biocatalyst.

Although physiological agents that are required in stoichiometrical quantities are not true enzymes, there is nevertheless a quantitative factor in enzyme action. The agent should not be consumed in the reaction if it is a true catalyst — or it should be returned as rapidly as it is withdrawn. But this is not entirely the case with the biocatalysts. They should convert unlimited amounts of raw material or substrate. They do not, owing to limitations of time or eventual inactivation or disappearance from the field of action. This limitation of the converting power may be likened to the "poisoning" of inorganic catalysts, which necessitates periodic rejuvenation. This situation, however, complicates the study of enzyme kinetics, which are important for the understanding of cell physiology and vital in industrial enzyme applications.

Reversibility of Enzyme Action: One aspect of enzyme reaction kinetics is characteristic, and probably not applicable to vitamins or hormones. Under the proper conditions, all enzymically accelerated reactions should be reversible. This has been proven in many instances, is commonplace with some enzymes, and would likely apply to all enzymically promoted reactions, if the proper conditions were known.

Enzymes are set apart in one or two other ways. When highly purified, they may need accelerators or co-enzymes. They are likely to be heat sensitive, which ties in with the generally accepted protein nature. Protein structure or the action of protein prosthetic groups may account for or control the degree of specificity in enzyme action — whether a phosphatase, for example, will hydrolyze a number of different phosphate esters, or only one.

Importance of the Phosphate Ester Enzymes

The phosphate ester enzymes were selected as the topic for this review for two reasons. They are undoubtedly the more important of the enzymes nutritionally, physiologically and pathologically. And a discussion of recent research on these enzymes will indicate the nature of the problems with which the enzymologist is currently occupied. The presentation will not attempt to be complete as to theory, nor can it include all of the published work. References were chosen rather as examples of lines of investigation.

In the early work of Harden and Young (2) on fermentation of sugar with zymase it was established that inorganic phosphate did not merely catalyze the degradation of sugar, but actually combined with it as a first step in the process. The enzyme responsible for the hydrolysis of the resulting hexose phosphate ester was given the name of phosphatase, in conformity with enzyme terminology. Similar esters, usually organic phosphates with some degree of biological activity, serve as the substrate or raw material for other phosphatase enzymes.

Acid and Alkaline Phosphatases: Phosphatases are listed by Harrow (3) under the general heading of esterases and are described as follows: "These enzymes (phosphatases) are present in various tissues and hydrolyze various esters of phosphoric acid. The phosphatases, of which there seem to be quite a number, are divided into two groups: alkaline phosphatase, with an optimum pH of from 8 to 10, found in intestinal mucosa, kidneys, liver, etc., and acid phosphatase, with an optimum pH of from 4 to 5, present in blood serum, etc."

Nutritional Aspects: Although the phosphatases are of paramount importance in metabolism, they have not yet been fully appreciated as entities in nutrition. Some dietary relationships have, however, been established.

The phosphatases do seem to have significance in pathology, and may possibly have therapeutic applications. The nutritionist will be interested in the wide range of distribution established for phosphatases and their general occurrence in the body, and in some of the relationships with hormones and vitamins. The phosphatases are linked with the action of amylolytic enzymes. Related enzymes, the phosphorylases, hydrolyze glycogen with the addition of phosphate. The key enzyme in glycolysis is the fraction of muscle tissue with adenosinetriphosphatase properties capable of releasing energy at high level.

As in many lines of biological research, it is difficult to make unqualified general statements about any of the enzymes. For example, acid phosphatase is indeed found in blood serum, but alkaline phosphatase is present in serum also. There may be several acid phosphatases in biological materials, with different pH optima. Or the enzymes so designated may be mixtures of acid and alkaline phosphatases in different proportions. As to whether any one phosphatase is apparently identical with another, one must ask whether they act on the same substrates, at the same rates and pH optima, since there is no recognized chemical means of identification. Esters commonly used include glycerophosphate, phenylphosphate, hexosephosphate, phenolphthalein phosphate, adenosinetriphosphate. One or more of these esters may be hydrolyzed by a given phosphatase.

Test Procedures: For testing unknown materials and fractions for phosphatase content, the use of phenylphosphate as substrate simplifies the analytical procedure. A method described for blood serum (4) will work with other materials. For acid phosphatase action, citric buffering is used, and for alkaline action, carbonate-bicarbonate. The index is the rate of liberation of phenol.

This and similar methods have been used to establish the presence of phosphatase in a variety of animal fluids and tissues, in fruits and vegetables, in grain products, in yeast, and in mold and bacterial cultures.

Pasteurization Index: It is common practice in the dairy industry to use the residual phosphatase activity of milk as an indicator of the degree of pasteurization. The enzyme may also be inactivated by souring. Reactivation of the phosphatase has been demonstrated (5) in experiments which demonstrate glycerophosphate splitting properties on the alkaline side as well as on the acid side.

Preparation from Animal and Plant Sources

Milk has been used as the source of phosphatase for the preparation of enzyme concentrate (6). The product, a brownish powder, displays enor-

mously high activity toward sodium glycerophosphate.

An alkaline phosphatase has been prepared from cow udders (7) and concentrated 7,000-fold, with recovery of a yellowish, gelatinous substance easily soluble in water. This material causes a rise in blood sugar when injected subcutaneously into dogs and rabbits.

Enzymes may be differentiated by using a different form of the substrate, as well as by using a different kind of substrate. In a study of the distribution of phosphomonoesterase and phosphodiesterase (8) it was found that blood serum, urine, sperm and prostate tissue contain phosphomonoesterases which hydrolyze monophenyl phosphate at pH 5.2, but of these four sources, only serum contains an important amount of phosphodiesterase capable of hydrolyzing diphenyl phosphate in acid solutions. Swine serum was reported to contain three phosphodiesterases with maximum activity at pH 7.3, pH 6.6 and pH 4.8 respectively.

In Animal Organs: Studies on relative distribution of acid and alkaline phosphatases in amphibians (9) showed a relatively high content in the liver, with lesser amounts in pancreatic, lung and intestinal tissue. Optimal pH levels were pH 5 for acid phosphatase of liver and pH 9.6 for alkaline phosphatase.

Alkaline phosphatase has been recovered consistently from the mucous membrane of the small intestine of the calf (10, 11, 12, 13). Glycerophosphate is split readily at pH 9.2, desoxyribonucleic acid fractionally. The enzyme solutions can be concentrated by electrophoresis.

Dog liver (14) and hog kidney (15) have been used as sources of alkaline phosphatase. The phosphatase level in the male genital tract, normally high, may have some significance in pathology. Alkaline phosphatase was shown present (16) in the male rat in the vas deferens, seminal vesicles, prostate and Cowper's glands, but not in the testicle or epididymus. Castration resulted in total loss of phosphatase, then intramuscular injection of testosterone propionate caused the enzyme to reappear in normal amounts.

Phosphatase utilized in the preparation of ribonucleosides (17) was made from hypertrophic human prostate. The increasing adoption of artificial insemination in animal husbandry has suggested an examination of the phosphatase level of donor animals. The alkaline phosphatase content of the blood plasma of breeding bulls (18) was found to vary with sperm donation but it bears no relation to the plasma calcium, inorganic phosphate nor to age. Acid phosphatase was not subject to the same fluctuations.

In Plant Materials: Phosphatases are widely distributed in plant cells and tissues, especially in association with starch. A monophosphatase, active in alkaline medium, has been obtained from yeast (19). It acts strongly

on sodium phenolphthalein phosphate, phenyl phosphate and beta-glycero phosphate at pH 8.5 to 9.1.

Diastatic preparations from molds are known to display some phosphatase activity. Some of these are made from *Aspergillus oryzae.* From *Penicillium glabrum,* however, a phosphatase has been prepared which is 600 times as active as one of the commercial diastase products (20).

The enzyme activity of grains has been extensively investigated. In germinating barley, glycerophosphatase has been determined (21). In wheat milling products, using glycerophosphate (22), a decreasing content of enzyme has been found going down the mill stream as follows: shorts, bran, feed, clear flour, patent flour. This finding confirms histochemistry as to the distribution of phosphatase.

From white mustard seeds there has been recovered (23) a phosphomonoesterase which hydrolyzes beta-glycerophosphate but does not act upon inositol hexaphosphate.

Phosphatase from potato has been thoroughly studied (24). In the case of citrus fruits, the phosphatase content has been used as an index of juice pasteurization (25).

Histochemical Detection of Phosphatase

In studying the distribution and physiological significance of the phosphatases, considerable use has been made of histochemical techniques whereby the occurrence of active agents in the cellular content is revealed by appropriate sectioning and staining procedures. In one of these methods (26), sections of tissue are incubated with glycerophosphate. When the test is for acid phosphatase, lead nitrate is added also. When testing for alkaline phosphatase, calcium nitrate is added in place of lead nitrate. After incubation with the glycerophosphate substrate and the appropriate nitrate, the tissue is treated with a suitable stain, such as cobalt nitrate, which will reveal phosphate and hence show the site of any enzymic activity.

These and similar procedures may be sharpened to the point where closely related phosphatases may be distinguished in tissue. In an investigation of the different kinds of acid phosphatases in various cytological structures of the anterior pituitary of the guinea pig (27) it was concluded that at least two different acid phosphatases are present. These are effective at pH 6.6 and pH 5.5 and are supplementary in function.

Data on alkaline phosphatase are correlated (28) in an appraisal of techniques for determining the cytological position of alkaline phosphatase. Alkaline phosphatase was found in fixed preparations in sites in which it occurs under physiological conditions.

Hormone and Vitamin Control: Similar pinpointing showed hormone and vitamin control over the alkaline phosphatase level (29). Here the phosphomonoesterase level was revealed by "histochemical localization" in the digestive tract and kidney of the rat. The alkaline phosphatase disappears from the small intestine and kidney tubules after bilateral adrenalectomy but it reappears after the administration of large parenteral doses of riboflavin.

Other recent cytological work on phosphatase includes histochemical study of phosphatases in the mammalian pancreas and salivary glands (30), in pigeon brain (31) and in the cytoplasm of mast cells (32).

Pathological Significance: Cytological and pathological chemistry join hands in investigations of the possible relationship between phosphatase activity and disease, with the etiology of tumors, benign or malignant, a frequent target. Cytochemical studies have been carried out on mast cells in tissue and *in vitro* (33). Normal and tumor (benign mastocytoma) mast cells from the dog and tumor mast cells cultivated *in vitro* were found to contain acid and alkaline phosphatases in their cytoplasm. The tumor cells contained, in addition, acid and alkaline phosphatases in their nuclei.

Abnormal enzyme activity in cancers at various sites in the body seems to be definitely established (34). Histochemical technique was used to detect acid phosphomonoesterase in human neoplastic tissue, which, in section form, was allowed to act on sodium glycerophosphate substrate buffered to pH 5.0. It was found that carcinomata of the breast, lung, skin, bladder and all portions of the gastrointestinal tract show a characteristic increase in acid phosphomonoesterase activity in the tumors in comparison with their tissue of origin. The nuclei of all cells except prostatic epithelium and neurones react first in stained preparations. This predominant nuclear location of acid phosphomonoesterase in cancers of organs other than the prostate probably prevents its escape to the blood stream with the same facility as is seen in carcinoma of the prostate.

Explanation has been sought for the increase in the alkaline phosphatase activity frequently observed in the blood in cancer (35). It does not seem to be due to activation of the enzyme by magnesium since the blood magnesium shows no significant increase. High phosphatase level in blood serum in prostatic carcinoma may be lowered (36) by estrogen. Here both serum and hepatic phosphatase may be reduced.

With respect to the enzyme activity in the presence of neoplasms, it must be considered for the present that any relationship is concomitant, and that a causative relationship has not been demonstrated.

In Senility: A different but suggestive line of investigation is a study of acid phosphatase in the senile brain (37). The staining of brain tissue of

senile patients postmortem for acid phosphatase activity revealed deeper staining in senile plaques.

Phosphatase in the Formation of the Skeleton

Most of the foregoing physiological and clinical work has been concerned indirectly with the organic part of the substrate, usually the primary source of heat or energy. Considerable attention has, however, been paid to the inorganic part of the substrate, the phosphate which is essential for the formation of bone and tooth tissue. In a review of literature on phosphatase and the mechanism of ossification (38), it is concluded that the enzyme plays an important role in the calcification of bone and teeth "enabling the process to proceed at physiological speed."

Phosphatase has been demonstrated in bone marrow smears (39). Changes in the enzyme level in the blood serum in rickets have been followed (40), in work done on rats. Alkaline blood phosphatase determinations have been made on rachitic children receiving vitamin D_2 intramuscularly (41).

In Fractures: Paralleling the work on rickets, research has been done on enzyme activity in fractures. In one investigation, it was found that the phosphatase in a fractured bone was not increased significantly, but that in the blood plasma did increase significantly, attaining after 40 days about four times the normal level (42). Somewhat at variance with this finding is a report on phosphatase activity of the long bones during experimental fracture in the dog (43). Fracture of the tibia caused an increase in the phosphatase of the fractured bone and the adjacent metatarsals, but no increase in that of the intact tibia of the opposite leg.

A related paper describes the action of bone phosphatase on osteogenesis *in vitro* (44). Chick femora were cultured *in vitro*. The addition of the enzyme to the medium retarded growth and caused degenerative changes, although it is admitted that the results may be blamed on protein material.

In Tooth Formation: A dental publication carries a paper on alkaline phosphatase in pulpal tissue of human teeth (45). The phosphatase is present in young human teeth in which dentine formation and calcification are occurring.

Abnormal Diets and Phosphatase Levels

A number of investigators have concerned themselves with phosphatase levels under abnormal dietary conditions. During caloric restriction in young, older and parathyroidectomized older rats, there was a marked elevation in plasma alkaline phosphatase activity (46). This was thought due to altered carbohydrate metabolism rather than to defective bone metabolism.

With rabbits on a vitamin E deficient diet, changes were found in the phosphatase activity of muscle homogenates (47). Although the alkaline phosphatase activity was unaltered, there was a twofold increase in the acid phosphatase.

In the case of rats, a higher level of alkaline phosphatase was found in intestinal lymph than in plasma (48). The lymph activity decreases with fasting, increases after feeding.

Pregnant rats were the subject of a specialized study (49). Changes in the alkaline phosphate content of the mammary gland may be concerned with the synthesis of nucleoproteins during the growth phase and possibly with the synthesis of casein.

Rats were used also in a research on the relation between serum alkaline phosphatase and dietary essential amino acids (50). On a low protein diet, the rats showed an increased level of serum alkaline phosphatase as the growth rate decreased.

Dietary Components and the Origin of Enzymes

From the standpoint of dietetics, perhaps one of the most obvious questions is what elements, if any, in the food are related to the supply of enzymes needed for the normal metabolism of the body. In answering this question for the group of respiratory enzymes, one author (51) considers that deficiencies may occur in any of three ways: if vitamins such as thiamine, riboflavin, or niacin are not supplied; if adenylic acid is inadequate, which will interfere with coenzyme formation; if there is a lack of the amino acids essential to make the specific protein of the enzymes. In normal diet, with adequate B vitamins and meat (or the equivalent in nucleotides and amino acids) enzymes should be formed in the body as required. In the event of deficiencies, however, the needed elements may be therapeutically supplied.

Enzyme Kinetics

To round out this review of the phosphate enzymes and the variety of biochemical processes for which they are important, some recent work will next be mentioned on other enzymes and enzyme effects, including the kinetics of enzyme action and its reversibility; the occurrence and activity of phosphorylase, which is closely related to the phosphatase enzymes; the hydrolysis of starch and the nature of diastase; the course of glycolysis and the most recent ideas as to "quick energy" substances of muscle in which adenosinetriphosphatase acts as the trigger.

Although enzymic reactions are catalyzed by enzyme, they remain subject to the law of mass action. If the reaction products accumulate, the reaction

will slow down, frequently stopping far short of completion. In metabolic processes, however, the reaction products are usually removed rapidly, often with the assistance of several enzymes. When the products of hydrolysis are present in excess, the enzymic reaction may then be expected to go in the reverse direction. This reversibility of effect is especially significant in the case of the phosphate enzymes.

A recent review of the enzyme effect (52) discusses their mode of action, speed of enzyme reactions, function of coenzymes, enzymes in relation to vitamins and to certain of the elements.

Relationship to Protein

The behavior of enzymes is conditioned to some extent by their content of or dependence on protein or protein-related materials. This has physical and chemical consequences. To the extent they are colloidal, the surface energy relations must be considered (53), and will affect reaction rates. Qualitatively, however, the nature of the enzyme protein or associated protein may be related to the degree of specificity of the enzyme action. As to the protein nature itself, those enzymes thus far reported in the literature as crystalline and substantially pure are protein in nature. In this form, however, they may require for effective action coenzymes or other materials which are themselves inactive. In crude preparations, such activators are likely to be still present.

An incidental effect in protein metabolism has been reported with phosphoprotein. A phosphoprotein phosphatase found in mammalian tissues (54) will split inorganic phosphate from phosphoproteins without preceding proteolysis. The dephosphorylation is influenced by activators and inhibitors.

Phosphorylases Defined

Although phosphatase action has been shown reversible under proper conditions, the reversible enzyme reaction best known is probably that of phosphorylase. The phosphorylase enzymes are defined by Harrow (3) as "enzymes which can decompose polysaccharides as well as bring about the synthesis of the latter. For example, a phosphorylase obtained from muscle converts hexose-1-phosphate into a polysaccharide. The reaction is a reversible one. An x-ray diffraction pattern of this polysaccharide shows it to be similar in structure to starch obtained from plants. In contrast to this, when heart or liver phosphorylase is used in place of the one obtained from muscle, the polysaccharide obtained resembles glycogen."

Under the influence of the phosphorylase, the degradation of the complex compound, starch or glycogen, commences with the addition of phosphate. Farther along the same path, phosphate is removed by phosphatase

as has been previously discussed. The action of each type of enzyme may be reversed. The appropriate designation is apparent if the reactions are classified on the basis of what happens to phosphate while degradation — or utilization or fermentation — proceeds.

Phosphorylase may be as widely distributed in nature as is phosphatase, possibly more so. Preparation from potato and standardization of the enzyme have been described in detail (55). Potato phosphorylase is one form commercially available. Starch-synthesizing phosphorylase has been found on the cells of *Torulopsis rotundata,* a capsulated yeast (56).

In the preparation of highly purified phosphorylase from muscle, care is taken to exclude amylase and other enzymes (57). Here is an aspect of phosphorylase (and phosphatase) action which will be touched on later — the association of these enzymes with amylase and other enzymes of the diastase complex. The same point is considered in work on crystalline muscle phosphorylase (58).

Since enzymes are still known essentially by their effects, the question may be asked as to whether the same material could not exert phosphatase and phosphorylase action under respectively appropriate circumstances. The answer is a matter of terminology until such time as some chemical similarities or differences can be shown between the enzymic materials. There is at least one claim for differentiation or fractionation of phosphorylases (59). Three are described from animal sources, differing in the contained nucleotide configurations. A fourth, from plants, differs again in its lack of sensitivity to adenylic acid.

Insulin and Phosphorylation: Hormone-enzyme interrelation is confirmed in the case of phosphorylase in a paper (60) which further supports the close relationship of insulin to the process of phosphorylation of carbohydrates. Liver and muscle phosphorylase was shown reduced by a protein-poor diet in the case of white rats. The phosphorylase activity decreased 40 percent, a finding of importance in view of the chief role of the enzyme in the synthesis and hydrolysis of glycogen.

Phosphatases as Part of the Diastase Complex

Reference to recent literature on phosphorylase will draw the reader quickly into a study of all of the enzymes known to play a part in the formation and conversion of plant starch. "Diastase" is now well recognized as an enzyme complex, with certain individual enzymes exerting phosphatase and phosphorylase effects. The amylopectin and the amylose of the starch molecule are acted on differently by the diastase complex, and may be partially converted one to the other by enzymic action. In starch degra-

dation, the viscosity changes, clarity and stability of solutions, color with iodine and formation of reducing sugar are all indicators of the rate and kind of enzymic action. To explore this subject would lead far afield from the central theme of this review. Some of the more general conclusions may, however, be cited.

Starch and Glycogen Contrasted

A gross general difference between starch and glycogen appears in the conclusion (61) that starch seems to be formed by a one-way process and stored in the plant for a long time, whereas glycogen is formed as a reserve for short-term delivery and the amount is continuously changing.

In a study of starch synthesis and degradation it was shown (62) that there are carbohydrate activators in the synthesis of amylose by purified plant phosphorylase. Also, the agent from potato designated as Q-enzyme will catalyze the conversion of amylose into amylopectin (63). Q-enzyme action is related to the polymer form of starch components in work (64) on the Q-enzyme or cross-linking phosphorylase originally prepared as a lead (Pb) complex. With this material, it was possible to confirm the hypothesis that the action of Q-enzyme on amylose is to convert the long unbranched structure into the ramified structure of amylopectin without the concomitant liberation of reducing sugars. With this enzyme, the iodine-amylose color was reduced 90 percent, and amylopectin recovered from it in 74 percent yield, displaying the properties of amylopectin obtained from potatoes.

A sidelight on the diastase problem is the finding (65) of a factor in soybeans which enables starch hydrolysis to proceed farther at higher pH. In this instance, it is thought that the factor may be a phosphatase.

Trigger Effect in Muscular Energy

In order to deal more fully with the topic of glycogen — muscular energy and the trigger effect of phosphatase, there follows a digest in part of a paper by Cori (66) who with his associates has done extensive research in the field. The essentials:

> Enzymatic breakdown of glycogen leads to formation of blood sugar in the liver and lactic acid in the muscle. Various substances (insulin, epinephrin, ergotoxine, adrenocortical and anterior pituitary hormones) influence enzyme activity in the cell.
>
> Despite different end products of glycogen breakdown in liver and muscle, initial stages of degradation are the same in these two tissues and in other tissues as well. The first step in the breakdown of glycogen is a reversible enzyme process.

Limitations of *In Vivo* Experiments

Difficulties in research on this system include the handicap that *in vivo* experiments do not reveal the intermediate steps; they may not be recognizable in tissue extracts either; it is necessary to separate the enzymes, as by using specific poisons.

With glycogen phosphorylase from yeast, a rapid breakdown of glycogen occurs in the presence of inorganic phosphate and adenylic acid. Omission of the latter agents prevents the reaction from taking place. Of the two, the adenylic acid is a coenzyme.

The phosphorylase catalyzes the following reaction reversibly:

$$\text{Glycogen} + H_3PO_4 \rightleftharpoons \text{Glucose-1-phosphate}$$

At equilibrium, esterified phosphate amounts to 1/5 of the inorganic phosphate. The glucose-1-phosphate is, however, removed by phosphoglucomutase:

$$\text{Glucose-1-phosphate} \longrightarrow \text{Glucose-6-phosphate}$$

Phosphoglucomutase does not catalyze reversal of the reaction. The only other step in the formation of lactic acid not yet reversed anaerobically is the dephosphorylation of phosphopyruvic acid.

Liver, in contrast to muscle, contains a phosphatase which acts on the hexosemonophosphate formed from glycogen by the phosphorylase. The latter can be separated by adsorption on aluminum hydroxide. In dialyzed liver extracts, hexosemonophosphate and fermentable sugar form from the glycogen.

The combined action of phosphorylase and phosphatase explains blood sugar formation from glycogen in the liver.

The breakdown of phosphocreatine during muscular contraction is connected with the rephosphorylation of adenylic acid which is formed when adenosinetriphosphate (ATP) transfers its mobile phosphate groups to fructose-6-phosphate to form fructose-1, 6-diphosphate.

Role of Adenosinetriphosphatase: In the foregoing, the carbohydrate cycle is linked to the "quick-energy" material, adenosinetriphosphate, which with myosin, one of the muscle proteins, forms the mechano-chemical agent of muscular energy. This system, described in a previous review in this series (67) is generally accepted, although recent modifications include a differentiation of actomyosin from myosin, with the recognition that only part of the complex has adenosinetriphosphatase (ATP-ase) activity. Whether or not the enzyme and myosin are identical, the material which has this effect is one of the most interesting of all the phosphatases. It breaks the first phosphate linkage in ATP, a high-energy bond, forming adenosinediphosphate or ADP. From ADP a second phosphate can be released with high bond energy. The third phosphate, however, has low energy potential.

The ATP-ase (also known as adenylpyrophosphatase) is not limited to animals nor is its species specific. It has been prepared from potatoes (68). Prepared from snake venom (69) it split one mole of phosphate from one mole of ATP. Dialysis of the venom destroyed the activity, which could

then be restored by adding Mg or Ca ions.

A number of papers discuss the relationship between myosin and ATP-ase activity (70, 71). One of these (71) follows the ATP-ase activity in the young rat. It remains at a low level until the 16th day, then rises sharply. This rise coincides with muscle function. ATP-ase activity increases actually and relatively above the myosin content of the muscles. A converse result was shown (72) in nutritional muscular dystrophy. In both the hamster and the guinea pig, the muscle ATP-ase activity was appreciably lowered.

For a further treatment of the role of phosphate in carbohydrate utilization, the reader is referred to a review (73) which deals with high energy phosphate bonds, the phosphorylation of glucose, formation and breakdown of the glucoside bond, glycolysis, the fixation of CO_2, pyruvate oxidation and the tricarboxylic acid cycle, diabetes, and preparations and methods used in the study of these reactions.

Trend of Research

From this survey, it is apparent that the phosphatase enzymes are important for the growth, repair, nutrition and muscular functioning of the body throughout life. They play a role in the formation of bones and teeth. They are essential in the utilization, storage and mobilization of carbohydrate energy. They seem to be related to certain states of disease, especially of middle and later life. There are indications that some of the current research will be applied before long in dietary supplementation, and in therapeutics. The phosphatases are being used with greater frequency as tools in biological research, and they are becoming more generally available for this purpose.

As more is known of the phosphatase group, so will research intensify on other enzymes — a trend which has become definitely established. With this development will go a more complete understanding of the interrelations of the enzymes, the hormones and the vitamins.

REFERENCES CITED

The English titles of foreign language articles are from *Chemical Abstracts.*

1. RUSKIN, S. L. The role of the coenzymes of the B complex vitamins and amino acids in muscle metabolism and balanced nutrition. *Am. J. Digestive Dis., 13,* 110 (1946).
2. HARDEN, A. and Young, W. J. The alcoholic fermentation of yeast juice. *Proc. Roy. Soc. B,* 77, 405 (1906).
3. HARROW, B. Textbook of Biochemistry, Philadelphia, W. B. Saunders (1946).
4. BÁLINT, P., Komáromy, I. and Lenner, M. Determination of phosphatase in blood serum. *Orvosok Lapja es Népegészségügy 3,* 1233 (1947).

5. SJÖSTROM, J. The phosphatase enzyme and souring of milk. *Svenska Mejeritidn, 35,* 269 (1943); *36,* 119 (1944).
6. HANSSON, E., Solberg, P. and Sjöstrom, J. Manufacturing (production) of phosphatase concentrate from milk and some experiments on the effect of dilution on the phosphatase test. *Svenska Mejeritidn, 38,* 191 (1946).
7. KERPPOLA, W. Function of purified alkaline phosphatase and thymonucleic acid as its activator. *Acta Med. Scand. 130, Suppl. 206,* 412 (1948).
8. PLUMEL, M. Phosphodiesterases of blood serum. *Bull. Soc. Chim. Biol. 30,* 55 (1948).
9. PONZ, F. Digestive phosphatases in the amphibians. II. Liver, bile and pancreas. *Rev. Espan. Fisiol. 3,* 311 (1947).
10. v. EULER, H. and Fono, A. The phosphatase-splitting enzyme system of ribonucleic acid. I. The uniformity of the alkaline intestinal phosphatases. *Arkiv Kemi, Mineral. Geol. 25A, No. 23,* 12 pp. (1948).
11. v. EULER, H., Hahn, L. and Saluste, E. Animal phosphatases. *Arkiv Kemi, Mineral. Geol. 24A, No. 5,* 15 pp. (1946).
12. EK, B., v. Euler, H. and Hahn, L. Phosphatases of animal origin. *Arkiv Kemi, Mineral. Geol. 26B, No. 10,* 6 pp. (1948).
13. ZITTLE, C. A. Hydrolysis of ribonucleic and desoxyribonucleic acids with phosphoesterase from calf intestinal mucosa. Symposium on chemistry and metabolism of nucleic acids and their constituents. *Federation Proc. 6,* 488 (1947).
14. SARLES, H. Purification of liver phosphomonoesterases. *Compt. Rend. Soc. Biol. 141,* 1072 (1947).
15. CHERBULIEZ, E. and Bertagna, P. The alkaline phosphatase of hog kidney and the Mg + + P_2O_7 - - - - system. *Helv. Chim. Acta. 32,* 663 (1949).
16. SOULAIRAC, A. and Thibault, C. Activated phosphatase in the mammalian genital tract. I. Localization in the male rat and experimental methods. *C. R. 227,* 446 (1948).
17. REICHARD, P. Preparation of ribonucleosides from small amounts of ribonucleic acid. *J. Biol. Chem. 179,* 763 (1949).
18. REID, J. T., Ward, G. M. and Salsbury, R. L. Effects of certain factors upon the level of the plasma phosphatases of breeding bulls. *Am. J. Physiol. 152,* 280 (1948).
19. HOFFMANN-OSTENHOF, O., Moser, H. and Putz, E. A monophosphatase from yeast active in an alkaline medium. *Experentia 4,* 352 (1948).
20. FARRER, K. T. H. A phosphatase from *Penicillium glabrum. Australian J. Sci 12,* 31 (1949).
21. MASSART, L. Action of enzymes during the course of malting. *Fermentatio, 88,* (1946).
22. YIN, H. C. and Chou, P. C. Phosphatase activities of wheat milling products. *J. Chinese Chem. Soc. 15,* 186 (1948).
23. COURTOIS, J. Phytase. V. Preliminary study of the hydrolysis of glycerophosphates and inositol hexaphosphate. *Bull. Soc. Chim. Biol. 29,* 944 (1947).
24. HELFERICH, B. and Stetter, H. Potato phosphatase II. *Ann., 560,* 191 (1948).
25. AXELROD, B. Phosphatase activity as an index of pasteurization in citrus juices. *Fruit Products J. 26,* 132 (1947).
26. CAPPELIN, M. A technique for the cytochemical determination of phosphatases. *Bull. Histol. Appl. Physiol. et Path. et Tech. Microscop. 24,* 155 (1947).
27. ABOLINS, L. and Abolins, A. Different kinds of acid phosphatases in various cytological structures of the anterior pituitary of the guinea pig. *Nature, 164,* 455 (1949).
28. DANIELLI, J. F. A critical study of techniques for determining the cytological position of alkaline phosphatase. *Exptl. Biol. 22,* 110 (1946).
29. SOULAIRAC, A. Histochemical localization of alkaline phosphatase (phosphomonoesterase) in the digestive tract and kidney of the rat. *C. R. Soc. Biol. 142,* 645 (1948).
30. NOBACK, C. R. and Montagna, W. Histochemical studies of basophilia, lipase, and phosphatases in the mammalian pancreas and salivary glands. *Am. J. Anat. 81,* 343 (1947).
31. SINDEN, J. A. and Scharrer, E. Distribution of certain enzymes in the brain of the pigeon. *Proc. Soc. Exptl. Biol. Med. 72,* 60 (1949).
32. RILEY, J. F. and Drennen, J, M. The presence and significance of alkaline phosphatase in the cytoplasm of mast cells. *J. Path. Bact. 61,* 245 (1949).
33. PAFF, G. H., Montagna, W. and Bloom, F. Cytochemical studies of normal and tumor mast cells in tissue and in vitro. *Cancer Research 7,* 798 (1947).
34. LEMON, H. M. and Wisseman Jr., C. L. Acid phosphomonoesterase activity of human neoplastic tissue. *Science 109,* 233 (1949).
35. TRUHAUT, R. and Vermes, M. E. Magnesium and alkaline phosphatases of blood of cancer patients. *C. R. Soc. Biol. 141,* 717 (1947).
36. SHERLOCK, S. P. V. and Walshe, V. Hepatic alkaline phosphatase: histologic and microchemical studies on liver tissue in normal subjects and in liver and bone disease. *J. Path. Bact. 59,* 615 (1947).
37. JOSEPHY, H. Acid phosphatase in the senile brain. *Arch. Neurol. Psychiat. 61,* 164 (1949).
38. ROCHE, J. Phosphatase of bone and the general mechanism of ossification. *Experentia 2,* 325 (1946).

39. RABINOVITCH, M., Junqueira, L. C. U. and Mendes, F. T. Cytochemical demonstration of acid phosphatase in bone marrow smears. *Science 107,* 322 (1948).
40. DIKSHIT, P. K. and Patwardhan, V. N. Studies in experimental rickets: alkaline serum phosphatase in rachitic albino rats. *Indian J. Med. Res. 35,* 91 (1947).
41. MIGUEL, E. J. Alkaline blood phosphatase in rachitic Uruguayan children. *Anales Asoc. Quim. y Farm. Uruguay 50,* 63 (1948).
42. SOLERIO, L. and Sabaino, D. Bone and plasma phosphatase in fractures. *Arch. Sci. Med. 85,* 37 (1948).
43. FONTAINE, R., Chevallier, A., Mandel, P., Wiest, E. and Schuller, M. Phosphatase activity of the long bones during experimental fracture in the dog. *C. R. Soc. Biol. 142,* 702 (1948).
44. CAPPELLINI, M. Phosphatase action of bone phosphatase on bone osteogenesis in vitro. *Sperimentale 99,* 133 (1948).
45. BRUCKNER, R. J. Alkaline phosphatase in pulpal tissue of human teeth. *J. Dental Research 28,* 55 (1949).
46. HANDLER, P., Baylin, G. J. and Follis Jr., R. H. The effects of caloric restriction on skeletal growth. *J. Nutr.* 34, 677 (1947).
47. CAREY, M. M. and Dziewiatkowski, D. D. The adenosinetriphosphatase and phosphatase (acid and alkaline) activity of muscle homogenates from rabbits on a vitamin E-deficient diet. *J. Biol. Chem. 179,* 119 (1949).
48. FLOCK, E. V. and Bollman, J. L. Alkaline phosphatase in the intestinal lymph of the rat. *J. Biol. Chem. 175,* 439 (1948).
49. FOLLEY, S. J. and Greenbaum, A. L. Changes in the arginase and alkaline phosphatase contents of the mammary gland and liver of the rat during pregnancy, lactation and mammary involution. *Biochem. J. 41,* 261 (1947).
50. TUBA, J., Cantor, M. M. and Richards, G. A. Relation between serum alkaline phosphatase and dietary essential amino acids. *Can. J. Research 27E,* 25 (1949).
51. RUSKIN, S. L. The control of muscle spasm and arthritic pain through sympathetic block at the nasal ganglion and the use of the adenylic nucleotide. *Am. J. Digestive Dis. 13,* 311 (1946).
52. BUTLER, J. A. V. Enzymes. *J. Incorp. Brewers' Guild 34,* 392 (1948).
53. AFANAS'EV, P. V. The nature of enzyme activity. *Doklady Akad. Nauk S. S. S. R. 63,* 61 (1948).
54. FEINSTEIN, R. N. and Volk, M. E. Phosphoprotein phosphatase in mammalian tissues. *J. Biol. Chem. 177,* 339 (1949).
55. WEIBULL, C. and Tiselius, A. The starch phosphorylase of potato. *Arkiv Kemi, Mineral. Geol. A 19, No. 19,* 25 pp. (1945).
56. MAGER, J. Studies on the polysaccharides of capsulated yeasts. *Biochem. J. 41,* 603 (1947).
57. PETROVA, A. N., Rozenfeld, E. L., Lebedeva, L. B. and Spitsina, L. N. Preparation of highly purified muscle phosphorylase. *Doklady Akad. Nauk S. S. S. R. 66,* 1141 (1949).
58. HESTRIN, S. Action pattern of crystalline muscle phosphorylase. *J. Biol. Chem. 179,* 943 (1949).
59. KRITSKII, G. A. Prosthetic group of phosphorylase. *Doklady Akad. Nauk S. S. S. R. 64,* 373 (1949).
60. YAKOVLEV, N. N. Evolutionary explanation of the insulin regulation of carbohydrate metabolism in muscle. *Fiziol. Zhur. (J. Physiol.) 34,* 95 (1948).
61. MYRBACK, K. and Sillén, L. G. Amylopectin and glycogen: products of irreversible and reversible synthesis. *Nature 163,* 410 (1949).
62. BOURNE, E. J., Sitch, D. A. and Peat, S. Enzymic synthesis and degradation of starch. III. The role of carbohydrate activators. *J. Chem. Soc.* 1949, 1448.
63. BARKER, S. A., Bourne, E. J. and Peat, S. Enzymic synthesis and degradation of starch. V. The action of Q-enzyme on starch and its components. *J. Chem. Soc.* 1949, 1712.
64. PEAT, S. Bourne, E. J. and Barker, S. A. Enzymic conversion of amylose into amylopectin. *Nature 161,* 127 (1948).
65. PEAT, S., Whelan, W. J. and Pirt, S. J. Amylolytic enzymes of soybeans. *Nature 164,* 499 (1949).
66. CORI, C. F. Glycogen breakdown and synthesis in animal tissues. *Endocrinology 26,* 285 (1940).
67. WOODS, R. Modern concepts of food utilization. *These reviews 9, No. 5* (1948).
68. KIRSHNAN, P. S. The preparation of apyrase from potato. *Arch. Biochem. 16,* 474 (1947).
69. ZELLER, E. A. An adenosinetriphosphate splitting enzyme of snake venom. *Experentia 4,* 194 (1948).
70. SPICER, S. S. The effect of precipitation and dehydration on the physiologic and enzymatic properties of myosin and actomyosin. *Arch. Biochem. 25,* 369 (1950).
71. HERRMANN, H. and Nicholas, J. S. Enzymic liberation of inorganic phosphate from adenosinetriphosphate in developing rat muscle. *J. Exptl. Zool. 107,* 177 (1948).
72. HUMMEL, J. P. Oxidative phosphorylation processes in nutritional muscular dystrophy. *J. Biol. Chem. 172,* 421 (1948).
73. VENNESLAND, B. Carbohydrate metabolism. *Ann. Rev. Biochem. 17,* 227 (1948).